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(54) **Labelled, modified oligonucleotides.**

(57) The invention relates to novel labelled modified oligonucleotides useful for gene therapy, a conjugate of a targeting moiety, such as an antibody and a modified oligonucleotide complementary to the labelled oligonucleotide, the combination of both being useful in therapy of viral infections, tumours and (auto) immunodiseases. Also disclosed are pharmaceutical formulations comprising the various compounds.

EP 0 490 434 A1

The invention relates to modified oligo-nucleotides which are radioactively labelled.

Radioactively labelled oligonucleotides are known, for instance as probes used in hybridization assays. The use of such oligonucleotides in vivo generally will not be possible, because unmodified DNA and RNA are quickly metabolized in vivo. For in vivo purposes modified oligonucleotides have been described in E. Uhlmann and A. Peyman: Chemical Reviews, 90, 543 (1990).

In so-called "anti-sense oligonucleotide" therapy, synthetic, modified DNA or RNA fragments, which may or may not be conjugated with enzymes or cytostatics, are directed towards complementary nucleic acid fragments (DNA or RNA) which are present in the "target" cells, for example of tumours or cells infected by a virus. Modification of the anti-sense oligonucleotide is necessary for the desired stability in circulation (inter alia, they must be able to withstand endogenous enzymes) and, in addition, the penetration in cells and tissues can be improved as a result of the modification. The anti-sense oligonucleotide is complementary to a nucleic acid in the target cell and may inhibit DNA transcription, mRNA translation, or (viral) nucleic acid replication.

Up to now "anti-sense oligonucleotides" have not been conjugated with chemical groups which are labelled with radioactive isotopes which can be used in radioimmunotherapy.

The present invention relates to modified "anti-sense oligonucleotides" which are labelled with isotopes which can be used therapeutically (radioactive anti-sense nucleotide: RAN) and the use of RANs in anti-cancer and anti-viral therapy. Moreover, the RANs can be labelled in such a way that the localisation of the complementary nucleic acid (fragment) is taken into account. If the RAN has to have an intracellular action, isotopes which emit Auger electrons are preferred (such as ^{77}Br , ^{80}Br , ^{125}I and ^{123}I), while in the case of extracellular localisation alpha- or beta-emitting isotopes (such as ^{90}Y , ^{131}I , ^{186}Re , ^{211}At and Bi isotopes) are preferred. The latter category of isotopes is also preferred if the RAN has to reach its "target" both inside and outside the cell.

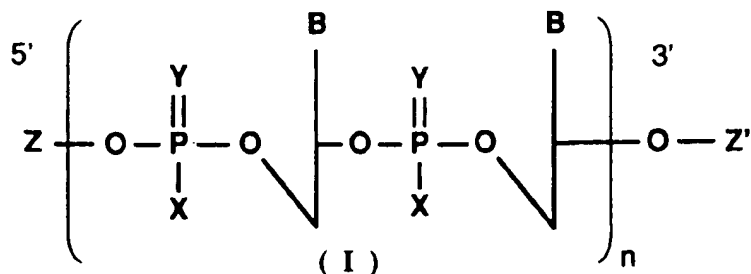
The RANs may be prepared according to any known technique for synthesis of oligonucleotides, for example as described in E. Uhlmann and A. Peyman: Chemical Reviews, 90, 543 (1990). Natural sugar backbones (ribose and deoxyribose) or their enantiomers may be chosen, possibly suitably modified, for instance 2'-O-methyl ribose, but it will also be possible to use stereoisomers (enantiomers α instead of β nucleosides) to choose other backbones such as deoxyglucose, as long as they can be made more resistant to endogenous enzymes and as long as they can provide for base pairing of the oligonucleotides, with a sufficient T_m (melting temperature).

Also the phosphate binding between the sugars may be replaced by a more stable bond (in vivo), such as $-\text{CH}_2-\text{SO}_2-\text{CH}_2-$, or $-\text{O}-\text{CH}_2-\text{O}-$.

The bases may be the natural bases (A,U,C,G,T), but they may also be rare bases such as xanthine, hypoxanthine and bases such as described in Benner et al., J.Am.Chem.Soc.Vol.111 p21,1989.

The RANs may be either linear oligonucleotides or circular oligonucleotides.

Preferred RANs are radioactively labelled, modified oligonucleotides of type I.



wherein:

$$n = 5 - 20$$

B = nucleoside base (C, G, A, T, U) or analogous bases, optionally labelled with a radioactive halogen.

X = O⁻, S⁻, N-dialkyl, O-alkyl, alkyl and/or combinations thereof.

Y = O and/or S.

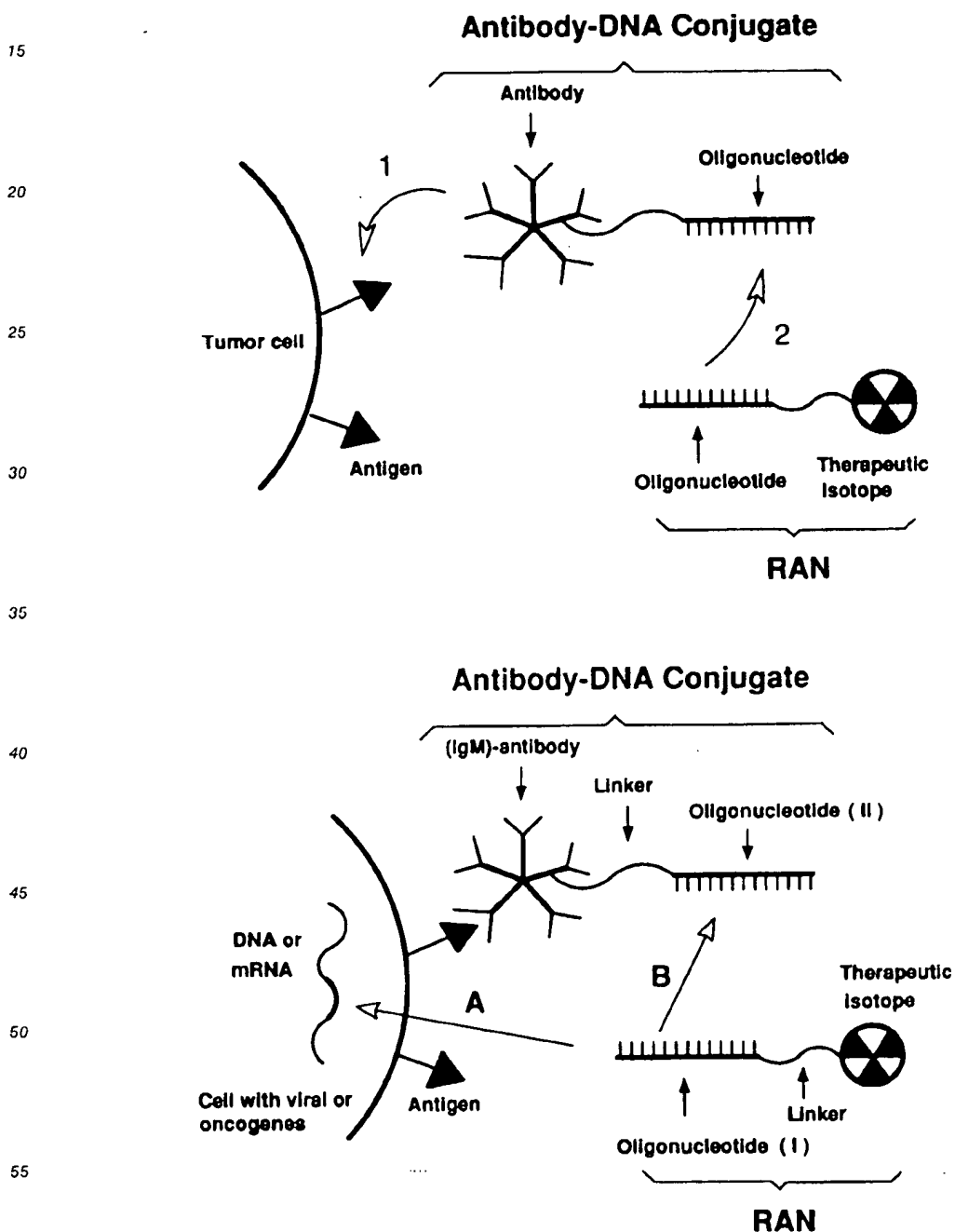
Z,Z' = a chemical group which contains a radioactive isotope, and/or Z,Z' is H, alkyl, acyl or nucleoside.

The base sequence (location of B) is determined by the base sequence of the "target nucleic acid fragment", the RAN being complementary to the sequence of the latter (for instance C-G and A-T as

complementary bases).

Another aspect of the invention provides a conjugate of a targeting moiety such as an antibody or a fragment or a derivative thereof, directed to an antigen associated with a target cell and a modified oligonucleotide which is complementary to a RAN. It is also possible to choose any other member of a specific binding pair which complementary member is associated with a specific group of target cells as a targeting moiety, such as ligands for receptors and the like. The conjugate can be administered to a subject; it will localize at the target site and after allowing sufficient time for said localization a RAN is administered, which will be bound by the complementary modified oligonucleotide in the conjugate. This will greatly enhance the possibilities of targeted therapy as used in for instance the combat of (auto) immune diseases, viral infections and tumours. Such a pretargeting programme is shown in scheme 1.

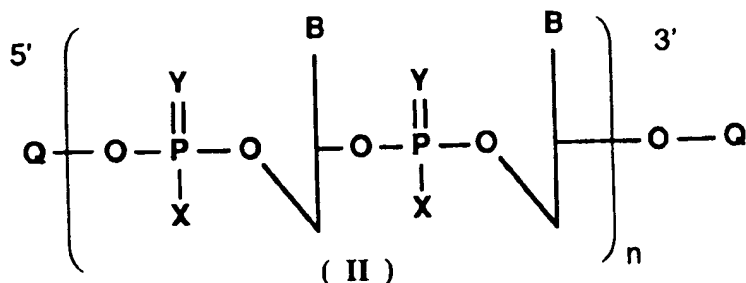
Scheme 1 A and B: Therapeutic applications of radiolabelled antisense nucleotides (RANs).



The use of specific antibodies in tumour therapy is known. However, the use of radioactively labelled antibodies is restricted by the slow uptake in the "target" tissues, as a result of which there is an unfavourable ratio of exposure to radiation between diseased and healthy tissues.

A so-called "pre-targeting programme" is used in order to improve this unfavourable ratio. In this programme non-radioactive bi-functional targeting moieties such as antibody conjugates are dispatched to the diseased tissues (initial binding) and subsequently, at the time when the antibodies are distributed in an optimum ratio between diseased and healthy tissues, a radioactively labelled ligand is administered which is distributed rapidly over the tissues and adheres to the second binding site of the antibody. However, possibilities for pre-targeting which are mentioned in the literature still have their limitations, inter alia as a result of, on the one hand, the low affinity of the second specific binding and, on the other hand, due to the use of substances foreign to the body, such as avidin (see Goodwin et al., J. Nucl. Med. 28, 722 (1987) and 29, 226 (1988)). The use of RANs has the advantage that RANs i) have a strong similarity to substances found in the body, ii) enter into strong, specific bindings, iii) distribute rapidly and well over tissues and iv) the residence time in the human body can be optimised by means of the nature of the chemical modification.

If RANs are directed towards oligonucleotides bound to targeting moieties such as antibodies, the bound oligonucleotide must be complementary to the RAN and of type II:



in which:

n = 5 - 20.

B = nucleoside base.

X = O⁻, S⁻, N-dialkyl, O-alkyl, alkyl and/or combinations thereof.

Y = O and/or S.

Q, Q' = a chemical fragment which is suitable for conjugation with targeting moiety. This fragment contains a reactive aldehyde, NH₂ or SH group, and/or Q, Q' is H, alkyl, acyl or nucleoside.

Such groups (Q, Q') and conjugation techniques which are needed to couple Q or Q' to targeting moiety are described in the review article by J. Goodchild: Bioconjugate Chemistry 1, pages 165 to 187 inclusive (1990).

Optionally the type II nucleotide may be labelled with an isotope for imaging.

It is preferred to couple several (type II) oligonucleotides to the targeting moiety, in order to increase the number of binding sites for the oligonucleotide of type I. The greatest effectiveness is achieved if the oligonucleotide sequence corresponds to a sequence which is unique for an intracellular oncogene or viral gene.

RANs are prepared by labelling chemically synthesized, modified oligonucleotides (type I) with the desired radioactive isotope. The synthesis of modified nucleotides which are suitable both for the RAN (type I) and for type II is effected in accordance with known methods such as described in Chemical Reviews 90 pages 543 to 584 inclusive (1990), modifications such as methylphosphonates, phosphorus amidates, phosphorus thioates and alkylphosphotri-esters being preferred, which - preferably - are incorporated in combination with naturally occurring phosphodiester bands.

Because the last mentioned oligonucleotides are usually prepared with the aid of so-called "solid phase" synthesis, the groups to be labelled are preferably positioned at the 5' end. Examples of these groups in the case of labelling with radioactive halogens are:

the highly charged oligonucleotides to positively charged blood proteins.

In principle, partially phosphate-methylated DNA fragments fulfill our goals as well. We were successful in developing a synthetic strategy for the preparation of well-defined partially phosphate-methylated DNA. The lability of the methyl phosphotriester in this modification requires mild conditions during conjugation and radiolabelling. Otherwise demethylation of the phosphotriester or other side-reactions may occur.

Example 1.

Using an Applied Biosystems DNA synthesizer (model 381A), the following complementary DNA fragments I and II, bearing 2 and 5 methylphosphonate linkages respectively, were synthesized (obtained after deprotection using ammonia in methanol during 3 days and purification with the aid of gel permeation chromatography)

I : U5' -5' Gp (Me) CCGGCGCAAGCGp (Me) C 3'

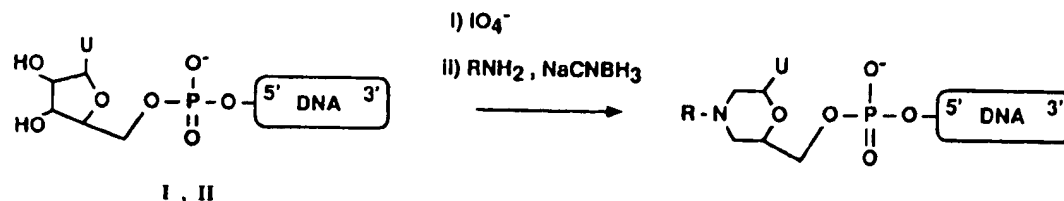
II : U5' -5' Gp (Me) CGCTp (Me) TGCGp (Me) CCGp (Me) Gp (Me) C 3'

p(Me) denotes a methylphosphonate which was introduced using suitably protected methylphosphonamides.

Duplex stability.

The stability of a duplex between two complementary DNA strands is expressed as the melting temperature T_m . This temperature, at which half of the helical structure is lost, can be monitored by measuring the absorbance at 260 nm as a function of temperature. The T_m -value for the DNA duplex between I and II in 0.1 M NaCl, 0.01 M Tris.HCl (pH = 7.0) was found to be 57 °C. Under physiological conditions in vivo (37 °C) an efficient hybridization will occur.

At the 5'-end of the modified DNA fragments (I, II) a 5'-5' linked uridine nucleotide was introduced. This uridine moiety offers, after oxidation of the cis diol, the possibility to incorporate various amino-containing linkers via reductive amination (Scheme 2). Obviously the highest modified DNA fragment II was used for conjugation with the antibody.



Scheme 2: Incorporation of amino-containing linkers via 5'-5' linked uridine.

Example 2.

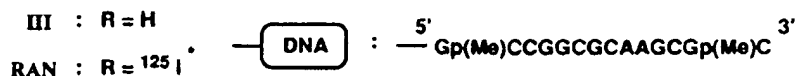
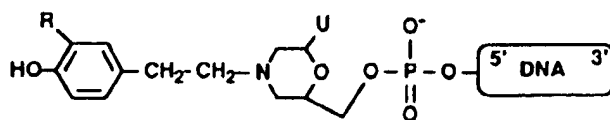
Preparation of radiolabelled oligonucleotides (RANs).

A RAN was obtained by radioactive labelling of the synthetic nucleotide (I).

U 5'-5'-Gp(Me)CCGGCGCAAGCGp(Me)C 3'

By reacting the oligonucleotide with sodium periodate (0.05 M ; 30 eq.) in sodium acetate buffer (0.1 M, pH = 4.75) during 1 hour at 0 °C in the dark, the ribose (of U) was converted to a dialdehyde. After removing the excess periodate on a Sephadex G-10 column, the dialdehyde was aminated in a solution of 0.05 M tyramine in 0.1 M phosphate buffer (pH = 6.9)/methanol (2:1 v/v).

After 30 minutes at room temperature sodium cyanoborohydride (0.1 M; 30 eq.) in methanol was added. The reduction was allowed to proceed overnight at room temperature, after which an additional amount (30 eq.) of sodium cyanoborohydride was added (1 hour, room temperature). The reaction mixture was concentrated and applied to a Sephadex G-25 column using 0.05 M TEAB as eluent. DNA-containing fractions were collected and lyophilized to yield the derivatized oligonucleotide III.



Scheme 3.

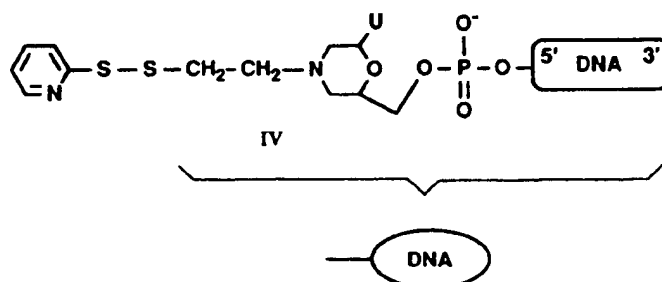
The p-hydroxyphenylethyl moiety of oligonucleotide III could be easily labelled with Na^{125}I in the presence of iodogen $^{\text{R}}$.

Briefly, an Eppendorf reaction tube was coated with iodogen $^{\text{R}}$ (50 μg) by drying an iodogen $^{\text{R}}$ solution in methylenechloride by means of nitrogen. To the reaction tube was added 10 μg Na^{125}I solution (≈ 2 mCi). The iodination was allowed to proceed for 15 minutes at room temperature with occasional shaking. The RAN was purified on Sephadex PD10 using either PBS buffer or 2 x SSC buffer (for hybridisation studies). The specific activity of the RAN thus obtained was 35 $\mu\text{Ci}/\mu\text{g}$.

Example 3.

Preparation of antibody-oligonucleotide conjugates.

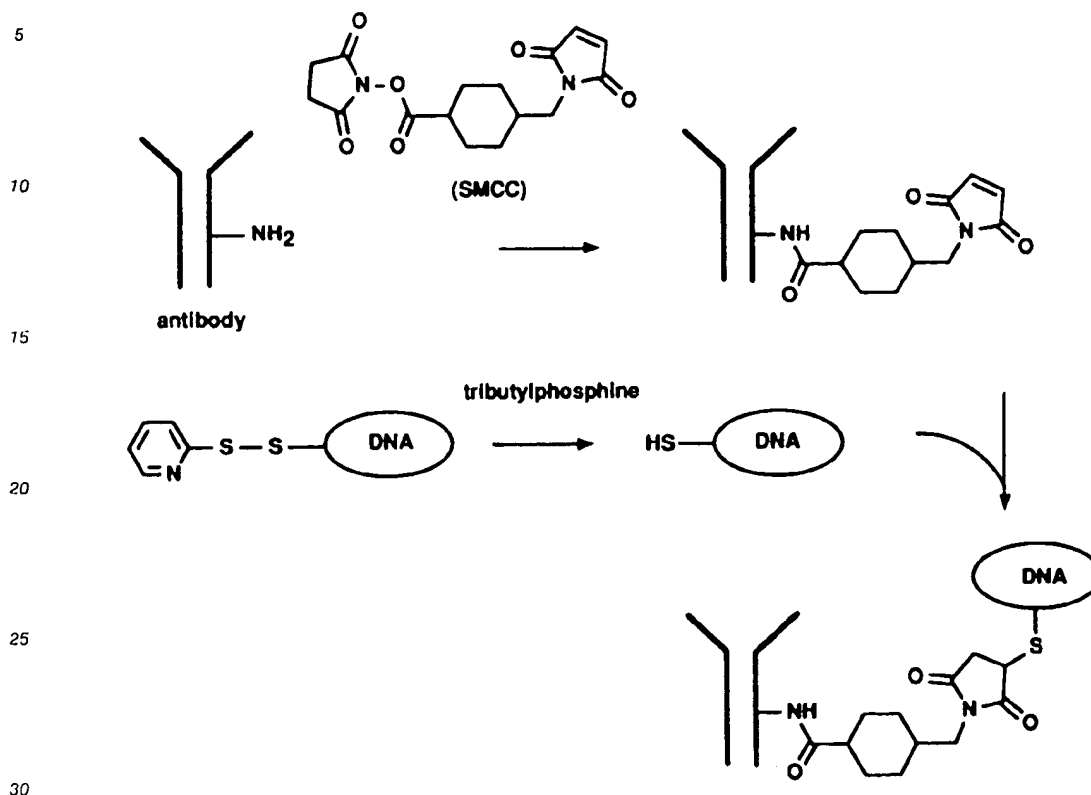
First, oligonucleotide II was derivatized with an activated thiol linker in an analogous way as described for the derivatization of oligonucleotide. Briefly, oligonucleotide II was oxidized with 0.05 M sodium periodate (12.5 eq.) in 0.12 M sodium acetate buffer (pH = 4.75) during 1 hour at 0°C in the dark. Subsequent to removal of excess periodate (Sephadex G-10) the dialdehyde was aminated with 0.04 M S-pyridylcysteamine. HCl (30 eq.) in 0.1 M phosphate buffer (pH = 8.0)/methanol (2:1 v/v), followed by reduction with sodium cyanoborohydride (5 eq.) in methanol during 16 hours at room temperature. An additional amount of sodium cyanoborohydride (5 eq.) was added before purification of the oligonucleotide on a Sephadex G-25 column using 0.05 M TEAB as eluent. Lyophilization of the DNA containing fractions afforded oligonucleotide IV containing a thiol linker activated as a 2-pyridyldisulfide. According to UV-absorbance of the pyridine-thione released upon reduction of the disulfide, approximately 45% of the oligonucleotide contained the activated thiol linker.



Scheme 4.

As disulfide linked protein-DNA conjugates may be insufficiently stable in in vivo systems, we focused on the maleimide coupling strategy. Generally, in this approach the antibody is derivatized with maleimide groups by treatment of the protein with succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC).

Subsequently, a conjugate with the thiol-containing oligonucleotide is formed via a stable thioether linkage (Scheme 5).



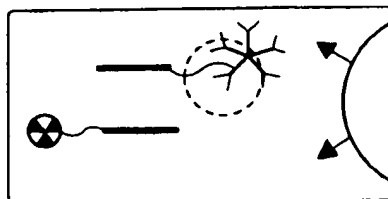
Scheme 5: Preparation of antibody-oligonucleotide conjugates.

Example 4.

In vitro experiments with the anti-HCG antibody 130A.

Initially, the anti-HCG antibody 130A was used to investigate the proper conditions for conjugation of the oligonucleotide.

Conjugate preparation. (I)



First, the IgG 130A in 0.05 M phosphate buffer ($\text{pH} = 7.5$) was reacted with SMCC (0.01 in DMF) during 1 hour at room temperature in the dark. An average of 10 maleimide groups per antibody was introduced upon treatment with 60 eq. of SMCC. The antibody was purified on a PD-10 column using 0.05 M phosphate/0.1 M NaCl/5 MM EDTA ($\text{pH} = 6.0$) as eluent. After reduction of the 2-pyridyldisulfide activated oligonucleotide in 0.1 M phosphate buffer ($\text{pH} = 8.0$)/methanol (2:1 v/v) was effected by treatment with tributylphosphine (1 eq., 5 min, r.t.), the thiol containing DNA fragment (50 eq. with respect to the antibody) was reacted immediately with the maleimide-derivatized antibody (Scheme 5). The conjugation

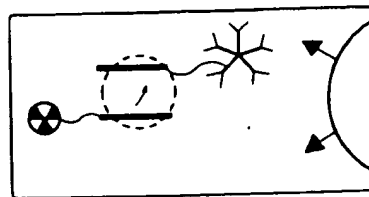
was allowed to proceed overnight at 4°C, followed by blocking of the unreacted maleimide groups with cysteamine.

The presence of covalently attached oligonucleotides in the conjugate preparation could be demonstrated by IsoElectricFocussing (IEF) and SDS-PAGE. In IEF a shift of the isoelectric point towards lower pH-value was observed, and SDS-PAGE showed an increase in molecular size in comparison with unconjugated IgG 130A.

The antibody-oligonucleotide conjugate could easily be separated from excess of unreacted oligonucleotide by gel filtration on Sephacryl S-100. HR column run at 4°C with PBS as eluent. The fractions containing antibody-conjugate were stored at 4°C.

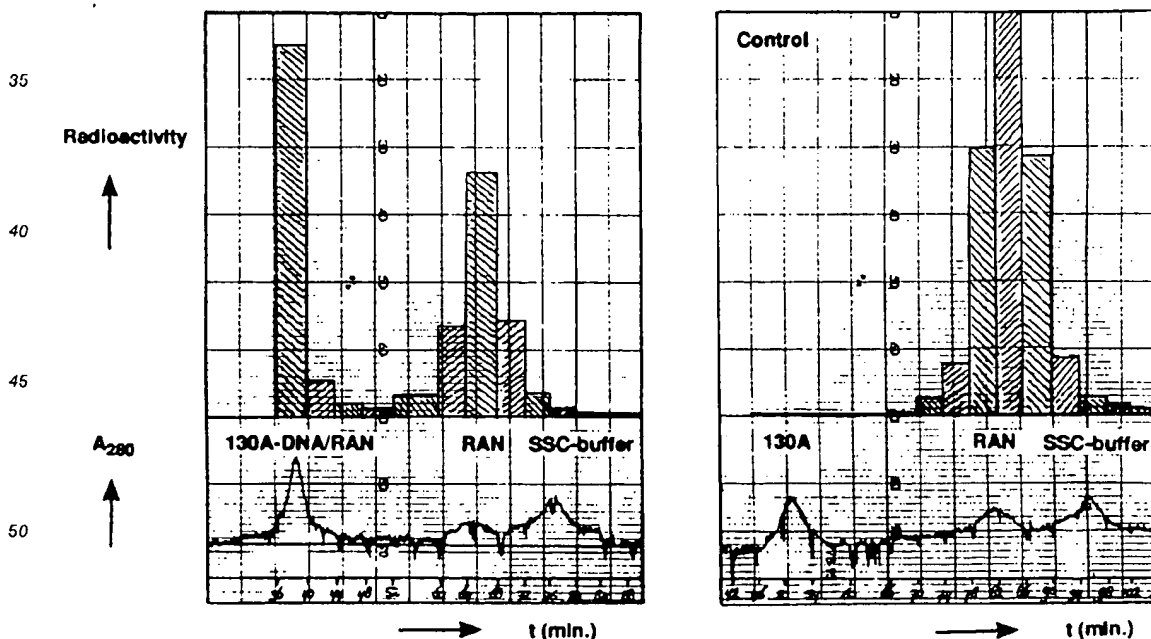
Based on the 260 nm/ 280 nm absorbance ratio of the purified conjugate a 3:1 molar composition of oligonucleotide and antibody was determined.

Hybridisation studies. (II)



The RAN was allowed to hybridise with the oligonucleotides of the antibody conjugate (10 µg/ml) in 2 x SSC buffer. A slight excess (approx. 1.2 eq.) of RAN was used in comparison with the total amount of conjugated DNA fragments. After 1 hr. hybridisation at room temperature, the mixture was applied on a Sephacryl S-100 column and eluted with PBS buffer at 4°C in order to separate unhybridised RAN from the conjugate/RAN complex. The absorbance at 280 nm and the radioactivity of the eluted fractions were measured (Scheme 6).

Scheme 6: Gel filtration of IgG130A-DNA/RAN hybridization mixture.



The elution profile showed radioactivity in both the conjugate peak and RAN peak. Approximately 45% of the eluted radioactivity was present in the peak containing the antibody-conjugate. In order to exclude non-specific binding of the RAN to the anti-body, a control experiment was carried out in

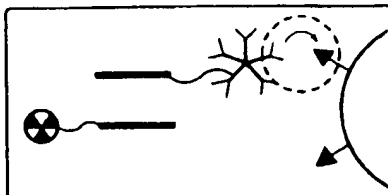
which underivatized IgG 130A was incubated with the RAN in the hybridization buffer. Gel filtration of the mixture revealed that less than 0.1% of the registered radioactivity was present in the antibody fraction.

These experiments demonstrate that the RAN is indeed capable to recognize the complementary sequence on the antibody via a specific hybridization mechanism.

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Antigen binding studies. (III)

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The next question to be answered was whether the antibody-oligonucleotide conjugate had retained its antigen binding properties. To elucidate this issue, part of the hybridization mixture (see above) was incubated with HCG-coated Sepharose beads.

20 After 90 minutes at room temperature, the mixture was centrifuged and the supernatant was removed. The beads were washed several times with hybridization buffer before the remaining radioactivity on the beads was determined. A 150-fold higher radioactivity was measured relative to the control experiment, in which HCG-Sepharose beads were incubated with a mixture of the RAN and IgG 130A and treated as above. This illustrates that no non-specific binding of the RAN to the antibody (or beads) occurs.

25 In conclusion, these antigen binding studies clearly indicate that the antibody after conjugation with the oligonucleotide has retained its ability to bind specifically to antigens in vitro.

Example 5.

30 In vitro experiments with the human anti-tumour antibody 1688.

Encouraged by the promising results with the IgG 130A, we focused our efforts on the IgM 1688, which, at the moment, is evaluated in radioimmunotherapy of various tumours.

35 IgM 1688 was activated with maleimide groups by treatment with SMCC as described for the 130A antibody. Since an IgM offers more reactive sites than an IgG, a slighter excess of SMCC was used in comparison with IgG 130A derivatization.

Reaction with 17 eq. of SMCC afforded an average derivatization with 8.5 maleimide groups, as was determined spectrophotometrically. The activated antibody was reacted immediately with freshly reduced (tributylphosphine) oligonucleotide IV (40 eq. with respect to the antibody). After conjugation overnight (4°C) and subsequent blocking of unreacted maleimide groups with cysteamine, the antibody-oligonucleotide conjugate was separated from excess oligonucleotide and other reagents on a Sephacryl S-100 HR column with PBS buffer as eluent at 4°C. Although a shift in the 260 nm/280 nm absorbance ratio was observed for the purified conjugate (0.63) in comparison with IgM 1688 (0.57), a precise quantification of the number of conjugated oligonucleotides was not possible, due to the dominating UV-absorbance of the protein. Based on our experience with the 130A antibody, however, we estimate that 2-3 oligonucleotides are linked to the antibody.

45 In analogy with the IgG 130A experiments, the IgM 1688-DNA conjugate was incubated with the RAN (approx. 2 eq. with respect to the conjugate) in 2 x SSC buffer to allow hybridisation of the complementary DNA fragments. After a hybridisation period of 1 hr. at room temperature, a S-100 column (PBS buffer, 4°C) was used to separate the antibody containing fraction from unhybridized RAN. Determination of radioactivity in both fractions revealed that 16% of the eluted counts was present in the antibody-conjugate fraction. As with IgG 130A, the control experiment, in which RAN and IgM 1688 were incubated and subsequently separated, showed that negligible non-specific binding of RAN to IgM 1688 had occurred: 0.2% of eluted radioactivity was present in the antibody peak.

50 These findings were confirmed by a series of dot-blot hybridisation experiments. In this assay a dilution series of the IgM 1688-DNA conjugate was immobilised on a nitrocellulose filter. After blocking the filter with BLOTTO to suppress non-specific binding, it was incubated with RAN during 2 hr. at room temperature in 2 x SSC buffer. Then, the filter was washed and imaged with autoradiography, showing a good dilution pattern.

In a parallel experiment, a similar dilution series of IgM 1688-DNA conjugate was treated with an equal amount of RAN diluted 100-fold with unlabelled oligonucleotide III. The radioactivity spotted in this series perfectly matched the pattern for 100-fold dilution in the first experiment. In the control experiment, in which a nitrocellulose filter immobilised with IgM 1688 was incubated with RAN, only background radioactivity was detected.

Summarizing, these results prove that despite of the huge dimensions of the IgM antibody the RAN is still capable to interact specifically with the conjugated oligonucleotides.

Antigen binding studies.

The antigen binding capacity of the IgM 1688-DNA conjugate was, as with the IgG 130A conjugate, examined by incubation of Sepharose beads coated with CTA-1, the cognate antigen of IgM 1688, with a hybridisation mixture of conjugate and RAN. In comparison with the control IgM 1688/RAN mixture 17.5-fold radioactivity on the CTA-coated beads was measured.

Apart from the above experiment, in which the RAN was hybridised with the conjugate before the latter had bound to the antigen, an additional test was carried out mimicking the pre-targeting "binding sequence". Thus, first CTA-coated beads were incubated with IgM 1688-DNA conjugate overnight at 4°C (step 1). After removal of excess conjugate and several washings, the beads were incubated with RAN during 2½ hr. at room temperature in 2 x SSC buffer (step 2). Likewise, a competition experiment was performed including hybridisation with the same amount of RAN, diluted 100-fold with unlabelled oligonucleotide III. Subsequent to removal of the supernatant and several washings, the remaining radioactivity on the beads was determined. The results of these experiments are summarized in Table 1.

Table 1.

step 1	step 2	relative radioactivity
IgM 1688-DNA	RAN	10.6
IgM 1688 (control)	RAN	1.8
IgM 1688-DNA	RAN / DNA(III) (1:100)	1.4
IgM 1688 (control)	RAN / DNA(III) (1:100)	1

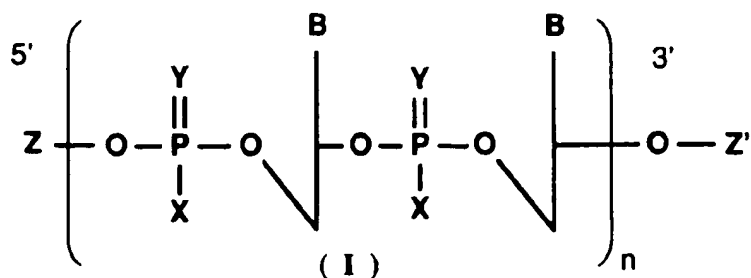
As expected, the highest radioactivity is found for the pre-targeting experiment with undiluted RAN. Moreover, the data clearly demonstrate the competitive behavior of the unlabelled oligonucleotides.

Above experiments (with CTA-coated beads acting as a model for antigen expressing tumor cells) illustrate that a radiolabelled oligonucleotide is able to recognize its complementary sequence on an antibody-conjugate, even when the latter is bound to its antigen. In this manner, pre-targeting based on DNA hybridization has been shown to be successful in vitro.

Human antitumour antibody 16.88 and its antigen are disclosed in EP(A) 0328578.

Claims

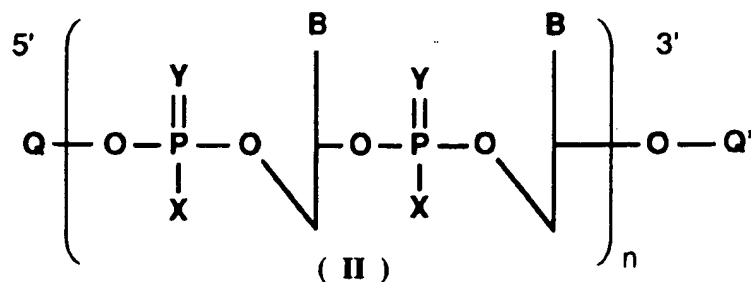
1. Labelled, modified oligonucleotide, characterised in that the oligonucleotide is complementary to a nucleic acid fragment which is localised in or close to a "target" cell and in that it is labelled with a therapeutic entity.
2. Labelled, modified oligonucleotide, characterised in that the oligonucleotide is complementary to a nucleic acid fragment which is localised in or close to a "target" cell and in that it is labelled with a therapeutic isotope.
3. Labelled, modified oligonucleotide according to Claim 2, characterised in that it has the general formula:



wherein:

- $n = 5 - 20$,
 $B =$ nucleoside base (C, G, A, T, U) or analogous bases,
 $X =$ O^- , S^- , N-dialkyl, O-alkyl, alkyl and/or combinations thereof,
 $Y =$ O and/or S,
 $Z, Z' =$ a chemical group which contains a radioactive isotope, or Z, Z' is H, alkyl, acyl or nucleoside,

4. A radiolabelled modified oligonucleotide according to claim 3, characterised in that B is labelled with a radioactive halogen.
5. Conjugate of a targeting moiety having specificity for a target cell and a modified oligonucleotide, characterised in that the oligonucleotide is complementary to a modified, labelled oligonucleotide according to one of Claims 1-4.
6. Conjugate according to Claim 5, characterised in that the modified oligonucleotide to be conjugated has the general formula:



in which:

- $n = 5 - 20$,
 $B =$ nucleoside base,
 $X =$ O^- , S^- , N-dialkyl, O-alkyl, alkyl and/or combinations thereof,
 $Y =$ O and/or S, and Q, Q' is a chemical fragment which is suitable for conjugation with an antibody.

7. A conjugate according to claim 5 or 6, characterised in that the targeting moiety is an antibody or a fragment or a derivative thereof.
8. Pharmaceutical composition for the elimination of specific cell populations, characterised in that it comprises a labelled, modified oligonucleotide according to one of Claims 1-4 and a suitable medium for administration.
9. Pharmaceutical preparation for the elimination of specific cell populations, characterised in that it comprises: a composition according to Claim 8, and a composition comprising a conjugate according to Claim 5, 6 or 7 and a suitable medium for administration.



European Patent
Office

EUROPEAN SEARCH REPORT

Application Number

EP 91 20 3183

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
X	WO-A-8 912 110 (BIOGEN INC.) * page 6; claims 1,6,26,46 *	1,2,8	C07H21/00 A61K31/70
A	---	7	
D,X	CHEMICAL REVIEWS. vol. 90, no. 4, June 1990, EASTON US pages 543 - 584; E. UHLMANN ET AL: 'Antisense Oligonucleotides: A New Therapeutic Principle' * page 573 - page 574 *	1,8	
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X	WO-A-8 906 702 (CHEMEX PHARMACEUTICALS INC.) * the whole document *	1,8	
X	---		
X	WO-A-8 905 853 (SYNTHETIC GENETICS) * the whole document *	1,8	
X	---		
X	WO-A-9 010 448 (GENETECH INC.) * the whole document *	1,8	
X	---		
X	WO-A-9 012 802 (UNITED STATES GOVERNMENT) * the whole document *	1,8	TECHNICAL FIELDS SEARCHED (Int. Cl.5)
A	---		
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A	WO-A-8 805 077 (BATELLE MEMORIAL INSTITUTE) * the whole document *	7,9	

The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 16 MARCH 1992	Examiner WATCHORN P.W.
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ----- & : member of the same patent family, corresponding document	

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